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1623

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March 27, 2002

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APR 02 2002

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Commissioner for Patents
Washington, D.C. 20231

Art Unit: 1623

Re: U.S. Utility Patent Application
Appl. No. 09/920,286; Filed: August 2, 2001
For: **Process for the Production of Multiple Cross-Linked
Hyaluronic Acid Derivatives**
Inventors: ZHAO
Our Ref: 0623.1110001/JMC/MGP

Sir:

Transmitted herewith for appropriate action are the following documents:

1. Claim for Priority Under 35 U.S.C. § 119 (a)-(d) in Utility Application including the following certified copy of the priority document cited therein: Great Britain Doc. No. GB 9902412.7; and
2. One (1) return postcard.

It is respectfully requested that the attached postcard be stamped with the date of filing of these documents, and that it be returned to our courier. In the event that extensions of time are necessary to prevent abandonment of this patent application, then such extensions of time are hereby petitioned.

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Commissioner for Patents

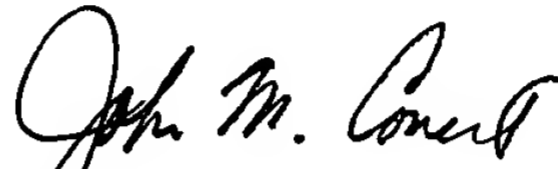
March 27, 2002

Page 2

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



John M. Covert
Attorney for Applicant
Registration No. 38,759

JMC/MGP/awt
Enclosures

P:\USERS\ATERRY\0623\111-1\pto cover letter priority

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re application of:

ZHAO

Appl. No. 09/920,286

Filed: August 2, 2001

For: **Process for the Production of
Multiple Cross-Linked
Hyaluronic Acid Derivatives**

Confirmation No. 3882

Art Unit: 1623

Examiner: *To Be Assigned*

Atty. Docket: 0623.1110001/JMC/MGP

**Claim For Priority Under 35 U.S.C. § 119(a)-(d) In Utility
Application**

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Commissioner for Patents
Washington, D.C. 20231

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Sir:

Priority under 35 U.S.C. § 119(a)-(d) is hereby claimed to the following priority document, filed in a foreign country within twelve (12) months prior to the filing of the above-referenced United States utility patent application:

Country	Priority Document Appl. No.	Filing Date
Great Britain	GB 9902412.7	February 19, 2002

A certified copy of each listed priority document is submitted herewith. Prompt acknowledgment of this claim and submission is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

John M. Covert
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Date: March 27, 2002

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I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

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Signed

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(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
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1. Your reference
JAF/P21420GB

2. Patent application number
(The Patent Office will fill in this part)
9902412.7 - 3 FEB 1999

3. Full name, address and postcode of the or of each applicant (underline all surnames)
FERMENTECH MEDICAL LIMITED

Research Avenue South
Heriot-Watt Research Park
Edinburgh EH14 4AP

Patents ADP number (if you know it)

75959760001

If the applicant is a corporate body, give the country/state of its incorporation

GB

4. Title of the invention
Process

5. Name of your agent (if you have one)
Kilburn & Strode

"Address for service" in the United Kingdom to which all correspondence should be sent
(including the postcode)

20 Red Lion Street
London
WC1R 4PJ

Patents ADP number (if you know it)

125001

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day / month / year)

7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application number	Date of filing (day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

a) any applicant named in part 3 is not an inventor, or

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body. See note (d))

Yes

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Priority documents	:	
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Statement of inventorship and right to grant of a patent (Patents Form 7/77)	:	
Request for preliminary examination and search (Patents Form 9/77)	:	
Request for substantive examination (Patents form 10/77)	:	
Any other documents (please specify)	:	

11. I/We request the grant of a patent on the basis of this application.

Signature Julia Florence Date 3 February 1999
Kilburn & Strobe

12. Name and daytime telephone number of person to contact in the United Kingdom

J A Florence - Tel: 0171-539 4200

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

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PROCESS

5 The present invention relates to a process for the production of hyaluronic acid (HA) derivatives, in particular cross-linked hyaluronic acid derivatives, and to novel cross-linked derivatives so obtained.

10 HA is a member of a class of polymers known as glycosaminoglycans. HA is a long chain linear polysaccharide and is usually present as the sodium salt which has a molecular formula of $(C_{14}H_{20}NNaO_{11})_n$ where n can vary according to the source, isolation procedure and method of determination. However, molecular weights of up to 14×10^6 have been reported.

15 HA and its salts can be isolated from many sources including human umbilical cord, rooster combs and nearly all connective matrices of vertebrate organisms. HA is also a capsular component of bacteria such as Streptococci as was shown by Kendall et al, (1937), Biochem. Biophys. Acta, 279, 401-405; it may therefore also be obtained by fermentation methods.

20 HA is non-immunogenic and therefore has great potential in medicine. Because of its visco-elastic properties HA having a high molecular weight (over 1 million) has been found to be particularly useful in a variety of clinical fields, including wound treatment, ophthalmic surgery and orthopaedic surgery. HA is also potentially useful in a variety of non-medical fields, such as cosmetic applications.

25

However, the use of HA in certain of these applications is limited by the fact that following administration to humans HA is readily degraded by enzymes

such as hyaluronidases. Furthermore, HA is soluble in water at room temperature, which can also make it less suited to certain applications. Various attempts have therefore been made to prepare more stable forms of HA, in particular by cross-linking the HA molecules.

5

Thus, USP4,582,865 (Biomatrix Inc.) describes the preparation of cross-linked gels of hyaluronic acid which are formed by cross-linking HA either by itself or mixed with other hydrophilic polymers using divinyl sulfone as the cross-linking agent. It appears that in this case the cross-linking occurs via the hydroxyl groups of HA.

10

USP5,550,187 (Collagen Corporation) describes a method for preparing cross-linked biomaterial compositions which involves mixing a biocompatible polymer, which is preferably collagen but may be selected from other polymers including hyaluronic acid, with a sterile dry cross-linking agent such as a synthetic hydrophilic polymer.

15

USP5,578,661 (Nepera Inc.) describes a gel forming system for use as a wound dressing which is formed from three main components, the first being a water soluble polymer, the second being an acid-containing polymer and the third being a polysaccharide or amino-containing polymer such as hyaluronic acid. In this case the cross-linking appears to be via ion-bonding.

20

USP5,644,049 (Italian Ministry for Universities and Scientific and Technology Research) describes a biomaterial comprising an inter-penetrating polymer network (IPN) wherein one of the polymer components is an acidic polysaccharide such as hyaluronic acid and the second polymer component may

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be a synthetic chemical polymer. The two components may be (but are not necessarily) cross-linked.

Tomihata and Ikada have reported cross-linking of HA using a water soluble carbodiimide as cross-linking agent. It was postulated that cross-linking took place via ester groups. The cross-linking reaction was also carried out in the presence of L-lysine methyl ester, which appeared to give additional cross-linking via amide bonds to the lysine ester. (J.Biomed.Mater.Res., 37, 243-251,1997).

US Patent No 5,800,541 describes collagen-synthetic polymer matrices prepared using a multiple step reaction. The first step involves reacting collagen with a synthetic hydrophilic polymer; the resulting matrix may then be modified in a second reaction step which may involve cross-linking or conjugating the matrix with a synthetic polymer, coupling biologically active molecules or glycosaminoglycans to the matrix, cross-linking the matrix using conventional chemical cross-linking agents or modifying the collagen in the matrix by means of chemical reaction. In this process, the initial collagen-synthetic polymer matrix appears to be cross-linked via only one type of bond, and the additional process steps serve to introduce further chemical substances which may form different types of bonds. However, it does not appear that any two of the substances forming the product will be linked to each other by more than one type of bond.

We have now found that if hyaluronic acid is cross-linked in two stages, such that cross-linking is first effected via one type of functional group, for example hydroxyl groups and then via a different functional group e.g. carboxyl groups,

a high degree of cross-linking is achieved with improved biostability of HA.

The present invention therefore provides a two-stage process for the preparation of cross-linked derivatives of hyaluronic acid.

5

The two-stage process according to the invention comprises:

- (a) cross-linking HA via a first functional group and subsequently
 - (b) further cross-linking the product of (a) via a second functional group,
- 10 wherein said first and second functional groups represent different chemical entities.

15 The functional groups which are mainly responsible for cross-linking of HA molecules are the hydroxyl and carboxyl groups. However, if desired the HA may be chemically modified prior to cross-linking to form other chemically reactive groups. Thus for example HA may be treated with acid such that it will undergo at least partial deacetylation, resulting in the presence of free amino groups.

20 It will be appreciated that the cross-link formed in the first stage of the reaction should be sufficiently strong to withstand the reaction conditions needed to form the second cross-link. Thus, the stronger of the two bonds should be formed first. This will be readily apparent to the skilled worker and if necessary can be determined by means of routine experimentation.

25

Thus, when the cross-links are to be formed via hydroxyl and carboxyl groups it will be recognised that the first-stage cross-linking should be effected via the

hydroxyl groups to give an ether linkage and the second-stage cross-linking will then be effected via the carboxyl groups, to give an ester link.

5 The two-step process can be achieved either by using a different cross-linking agent for each stage or by using the same cross-linking agent for both stages and adjusting the reaction conditions to control the specific cross-linking reaction required.

10 Thus, for example, an ether linkage may be formed under alkaline conditions, preferably at a pH of 10 or more, for example in the range of pH 10 to pH12. An ester linkage may be formed under acidic conditions, preferably at a pH of 4 or less, for example in the range pH 4 to pH2.

15 Cross-linking agents which may be used in the process of the present invention include those well-known in the art, for example formaldehyde, glutaraldehyde, divinyl sulfone, a polyanhydride, a polyaldehyde, a polyhydric alcohol, carbodiimide, epichlorohydrin, ethylene glycol diglycidylether, polyglycerol polyglycidylether, polyethylene glycol diglycidylether, polypropylene glycol diglycidylether, or a bis-or poly-epoxy cross-linker such as 1,2,3,4-
20 diepoxybutane or 1,2,7,8-diepoxyoctane.

To form an ether linkage the cross-linking agent is preferably selected from formaldehyde, glutaraldehyde, divinyl sulfone and, in alkaline conditions, bis and poly epoxides.

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To form an ester linkage the cross-linking agent is preferably selected from polyhydric alcohols, carbodi-imides, polyanhydrides, carboxylic acid chlorides

and, in acid conditions, bis and poly epoxides.

5 An amide linkage is preferably formed using a cross-linking agent selected from carbodi-imides in the presence of amines, carboxylic acid anhydrides and chlorides (with de-acetylated HA), and diisocyanates.

A sulfone linkage is preferably formed using a sulfonyl chloride.

10 The ratio of cross-linking agent to HA will generally be in the range of 0.5:1 to 10:1.

15 In order to achieve double cross-linking according to the present invention a first cross-linking reaction is carried out, for example using one of the methods described below. When this is complete, or has progressed as far as required, a further cross-linking agent is added to the reaction mixture. When this is the same cross-linking agent as employed in the first stage, the reaction conditions should be adjusted accordingly.

20 Thus, for example, a first cross-linking reaction to form an ether linkage will preferably be carried out under alkaline conditions, above pH 10 and a second cross-linking reaction to form an ester linkage may be effected, employing the same cross-linking agent, such as 1,2,7,8-diepoxyoctane, by adjusting the pH of the reaction medium to pH4 or less.

25 Alternatively a different cross-linking agent may be added, in which case it may not be necessary to adjust the reaction conditions. The second-stage cross-linking reaction is then allowed to proceed as for the first.

The individual cross-linking reactions may be carried out according to methods known generally in the art.

5 Thus, the HA utilised as the starting material may be in the form of a film or in solution. When HA film is employed, this may be suspended in a suitable solvent together with a cross-linking agent. The reaction medium preferably comprises an organic solvent such as chloroform, an alcohol e.g. ethanol, dimethylformamide or dimethylsulfone, admixed with an aqueous acidic or
10 alkaline solution. An acidic solution preferably has a pH of 4 or less and an alkaline solution preferably has a pH of 10 or above. The cross-linking reaction suitably takes place at a temperature in the range of 15 to 30°C e.g. ambient temperature. This method provides cross-linked HA in the form of a film.

15 Alternatively, HA may be employed as an aqueous acidic or alkaline solution to which the cross-linker is added. Under acidic conditions the pH of the starting solution is preferably pH4 or lower and for an alkaline solution the pH is preferably pH10 or above. The concentration of HA is suitably in the range 1 to 10% w/w. The reaction may be effected at a temperature in the range of 15 to
20 50°C. The time for completion of the cross-linking reaction may in general vary from about an hour to a few days. This method results in cross-linked HA in the form of a gel.

Whichever cross-linking method is used, the completion of the reaction can be
25 routinely determined by methods well known in the art.

The final product may be isolated from the reaction medium by conventional

procedures. Cross-linked HA prepared according to the present invention contains at least two different types of cross-linking bonds, for example both ether and ester bonds. This has been found to reduce the water absorption capacity of the cross-linked HA, resulting in greater stability in aqueous solution. In addition double cross-linked HA has been found to exhibit greater stability against degradation by hyaluronidase, indicating an increased biostability.

It is believed that cross-linked HA prepared according to the present invention is itself novel. Thus, in a further aspect the present invention provides cross-linked HA obtainable by the process described hereinbefore.

In a further aspect the present invention provides HA cross-linked to itself wherein the HA is crosslinked by at least two different types of bond.

The invention will now be further illustrated by the following non-limiting examples.

Example 1 – (Film)

5 ml of HA (1%) was cast for 4 days at room temperature in a fume-cupboard to get HA film. The resulting film was suspended in a mixture of CHCl_3 solvent/acidic or alkaline solution / 1,7-diepoxyoctane or glutaraldehyde cross-linker. The cross-linking reaction was effected at room temperature for a fixed time (24hr). A further amount of cross-linking agent was added, and if necessary the pH adjusted, and the mixture was allowed to stand at room temperature for a further 24 hours, to effect the second cross-linking reaction.

The detailed cross-linking conditions are shown in Table 1. After the cross-linking, the samples were washed with IPA and acetone for three times, immersed into IPA/DIW (60/40) overnight and then washed with acetone and dried in a 37°C oven to get a constant weight.

5

Example 2 – (Gel)

0.1g of HA was dissolved in 0.25N NaOH solution or 0.25N HCl solution to obtain HA solutions at 10% or 2.5% concentration. Cross-linking agent was added. The first cross-linking reaction was effected at room temperature or 40°C and the total cross-linking time varied from an hour to a few days. A second cross-linking reaction was effected using a further amount of the same cross-linker, with adjustment of the reaction conditions. Detailed reaction conditions are given in Table 2. After cross-linking, the formed gel was washed with IPA, acetone and extracted with IPA/water overnight and then washed with IPA and acetone respectively for three times. The samples were dried in a 37°C oven to achieve a constant weight.

10

Example 3

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0.1gm HA were dissolved in 2ml 1N NaOH solution overnight to get 5%HA alkaline solution. To this solution was added 0.2ml 1,2,7,8-diepoxyoctane. 0.2ml chloroform was then added whilst stirring at 40°C for 30 minutes. After the ether cross-linkage, 2.2ml 1N HCl was added to change the pH of the solution to between 3-4. A further 0.2ml 1,2,7,8-diepoxyoctane was added and 0.2ml chloroform was then added whilst stirring at 40°C for 30 minutes. After the ester cross-linkage, the formed gel (sample CHA-19) was precipitated with

25

20ml acetone and purified according to the same procedure as detailed in Example 2.

Example 4

5 To 5ml HA/NaOH (1N) solution, 0.5 ml epichlorhydrin and 0.2ml chloroform were added and mixed at room temperature for 10 minutes. The solution was cast in a petri dish and allowed to dry as a film of cross-linked HA (CHA-17). After neutralisation with 1N HCl, the CHA-17 sample was suspended in 20ml
10 chloroform/0.1N acidic aqueous solution (3/1 v/v)

0.2ml 1,2,7,8-diepoxyoctane was added and allowed to react at room temperature for 24 hours. The resulting sample, CHA-18, was purified according to the same procedure detailed in Example 1.

15

Table 1 Formation of Cross-linked HA (CHA) from HA Film

	First Cross-linker	Second Cross-linker	Time(h)	Temperature °C	pH
CHA-1	Glutaraldehyde	-	24h	RT	H+
CHA-2	Glutaraldehyde	Epoxide	24h/24h	RT	H+
CHA-3	Glutaraldehyde	Epoxide	24h/24h	RT	H+/OH-
CHA-4	Epoxide	-	24h	RT	H+
CHA-5	Epoxide	Glutaraldehyde	24h/24h	RT	H+
CHA-6	Epoxide	Epoxide	24h/24h	RT	H+/OH-
CHA-7	Epoxide	-	24h	RT	OH-
CHA-8	Epoxide	Epoxide	24h/24h	RT	OH-/H+

CHA-9	Epoxide	Glutaraldehyde	24h/24h	RT	OH-/H+
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Table 2 – Formation of Cross-linked HA (CHA) from HA Gel

	First Cross-linker	Second Cross-linker	Time (h)	Temperature°C	PH
CHA-10	Epoxide	Epoxide	2h/2h	40	OH-/OH-
CHA-11	Epoxide	Epoxide	2h/2h	40	OH-/H+
CHA-12	Epoxide	Epoxide	2h/2h	40	H+/OH ₋
CHA-13	Epoxide	Epoxide	2h/2h	40	H+/H+
CHA-14	Epoxide	-	72h	RT	OH-
CHA-15	Epoxide	-	72h	RT	H+
CHA-16	Epoxide	-	48h	RT	OH-
CHA-17	Epoxide	Epoxide	48h/24h	RT	OH-/H+

- 5 CHA-1, CHA-4, CHA-7, CHA-14, 15 and 16 were all prepared using a single cross-linking step, for comparative purposes.

H+ represents a pH of about 4

OH- represents a pH of about 10

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